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Pretreatment of guinea pigs with lysozyme prior to vaccination with the phase I antigen of Coxiella Burnetii enhanced antibody response and protection against challenge. An observed effect on macrophage migration suggests that the role of lysozyme includes stimulation of cell-mediated immunity.

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# Effect of Lysozyme on the Immune Response of Guinea Pigs to the Soluble Phase I Antigen of *Coxiella burnetii*

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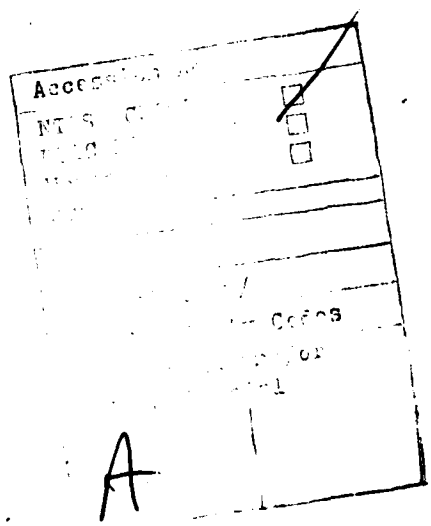
In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

## SUMMARY

Pretreatment of guinea pigs with lysozyme prior to vaccination with the phase I antigen of Coxiella burnetii enhanced antibody response and protection against challenge. An observed effect on macrophage migration suggests that the role of lysozyme includes stimulation of cell-mediated immunity.

In tests to determine the effect of enzymes on the properties of the soluble phase I antigen of Coxiella burnetii, we observed that guinea pigs vaccinated with lysozyme-treated antigen had higher antibody titers and were more resistant to Q fever challenge than those given untreated antigen. (R. F. Wachter and G. P. Briggs, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, E103, p. 71). Continued investigation suggested that the enhancement of immunogenicity might be attributed to an adjuvant action of lysozyme rather than to enzymatic modification of the antigen. Results presented in this report suggest that lysozyme may increase protection by influencing both cellular immune and humoral responses.

In a series of five similar, but not identical, experiments, guinea pigs (8 or 10/group for a total of 92) were inoculated subcutaneously (s.c.) with two doses, 14 days apart, of antigen only or of lysozyme followed by antigen 4 or 5 h later. Saline and lysozyme control groups (a total of 64 guinea pigs) were included. In some additional tests we varied the time of administration of lysozyme relative to antigen. A dialyzed trichloroacetic acid (TCA) extract of concentrated, partially purified phase I C. burnetii, Henzerling strain, was employed as the antigen. Lysozyme (3 x crystalline egg white, Sigma Chemical Co.) was administered at both the first and second dose interval. Doses of antigen ranged from 2 to 14 µg of protein, as determined by the Lowry method (6); doses of lysozyme ranged from12.5 to 250 µg. Doses used in each test are listed with Figure 1. Serum samples collected 14 days after the second inoculation were assayed for antibody by the microagglutination (MA) (2) and complement-fixation (CF) (1) tests. Guinea pigs were challenged intraperitoneally 28 to 45 days after the second dose with $5 \times 10^5$ median infectious doses of phase I C. burnetii.

Temperatures were recorded once daily for 10 days; animals with temperatures 40.0°C for two or more consecutive days were considered unprotected.

The effect of pretreatment of guinea pigs with lysozyme on protection against Q fever by the phase I antigen is indicated in Figure 1. Data shown represent the mean of five tests. Doses of antigen and lysozyme employed in individual tests are listed. No optimal dosage combination was found; lower dose levels were as effective as higher levels. Fifty-nine percent of 46 guinea pigs that received antigen only were protected compared to 83% of 46 that received lysozyme prior to antigen ($P < 0.02$). The time of administration of lysozyme relative to antigen appeared to be important: in a single experiment, lysozyme injected 24 or 48 h before antigen reduced the level of protection. In several other tests, administration of lysozyme and antigen at the same time, but at different sites, or of a mixture of lysozyme and antigen, either had no effect or reduced protection. Since this did not confirm our earlier observation with enzymatically-treated antigen (mentioned above), perhaps in the earlier enzyme experiments in which the lysozyme-antigen mixture was incubated at 37°C for 18 h, enhanced immunogenicity resulted from enzymatic alteration of the antigen, or from a combination of adjuvant effect of lysozyme with modified antigen.

For the same guinea pigs referred to in Figure 1 the effect of lysozyme on antibody response was determined on sera collected 14 days after the second dose. Figure 2 shows the geometric mean titers and percent animals responding for phase I and II MA antibodies and phase II CF antibody (phase I CF antibody is not produced at detectable levels from immunization with the phase I antigen). The doses for individual tests are the same as listed with Figure 1. The most pronounced difference

was seen with phase II CF antibody ($P < 0.001$); differences for MA-I and MA-II antibody titers were also significant, $P < 0.05$ and $P < 0.01$, respectively.

To investigate the possibility that lysozyme increased protection by stimulation of cellular immune mechanisms, we applied the macrophage migration-inhibition (MMI) technique to peritoneal cells from 4 groups of guinea pigs (4/group). Comparison was made between one group that received two doses, 2.0 and 6.0 µg (protein) of antigen only, 14 days apart, and a group that received lysozyme, 50 and 250 µg, 5 h before each dose of antigen. Peritoneal exudate cells were harvested, processed, and employed in the agarose droplet method of Harrington and Stastny (3) as applied by Kishimoto and Burger (4) to detect direct MMI. Cells were collected 4 days after intraperitoneal injection of 25 ml of sterile sodium caseinate. Half of the animals were started on test, i.e., given caseinate, one week, and half 2 weeks, after the second dose of antigen. In the absence of apparent differences, results from the 2 time periods were combined for purposes of analysis and presentation. Twenty replicate agarose droplets containing exudate cells were prepared from the cells harvested from each guinea pig. Subsets of 5 droplets each were overlaid with 0.2 ml of medium 199 (with calf serum) or with 0.2 ml of medium containing (a) 100 µg/ml lysozyme, (b) 20 µg/ml phase I antigen, or (c) 100 µg/ml lysozyme and 20 µg/ml antigen. Cultures were incubated, droplets examined, and migration inhibition calculated as described by Kishimoto and Burger (4).

The migration-inhibition of macrophages from guinea pigs that received antigen only (Fig. 3A) was much less than the inhibition of macrophages from animals that received lysozyme prior to antigen (Fig. 3B). Also, inhibition observed in subsets of droplets in the test system where

lysozyme plus antigen were employed as additives was substantially greater then in subsets with antigen alone; this was especially pronounced with macrophages from guinea pigs that received the lysozyme-antigen regimen. Also in this group, lysozyme itself produced limited inhibition.

Active immunity to Q fever has been reported to depend on both humoral and cellular responses (5). Other recent research has indicated that cellular immune mechanisms are exclusively responsible for protection against Q fever (M. S. Ascher, P. B. Jahrling, D. G. Harrington, R. A. Kishimoto, and V. G. McGann, Submitted to Clin. Exp. Immunol., 1980). The increase in CF antibody and the effect on macrophage-migration, which we have observed, suggest that the role of lysozyme in enhancing protection in the guinea pig host against Q fever could include both a stimulation of humoral response and cell-mediated immunity.

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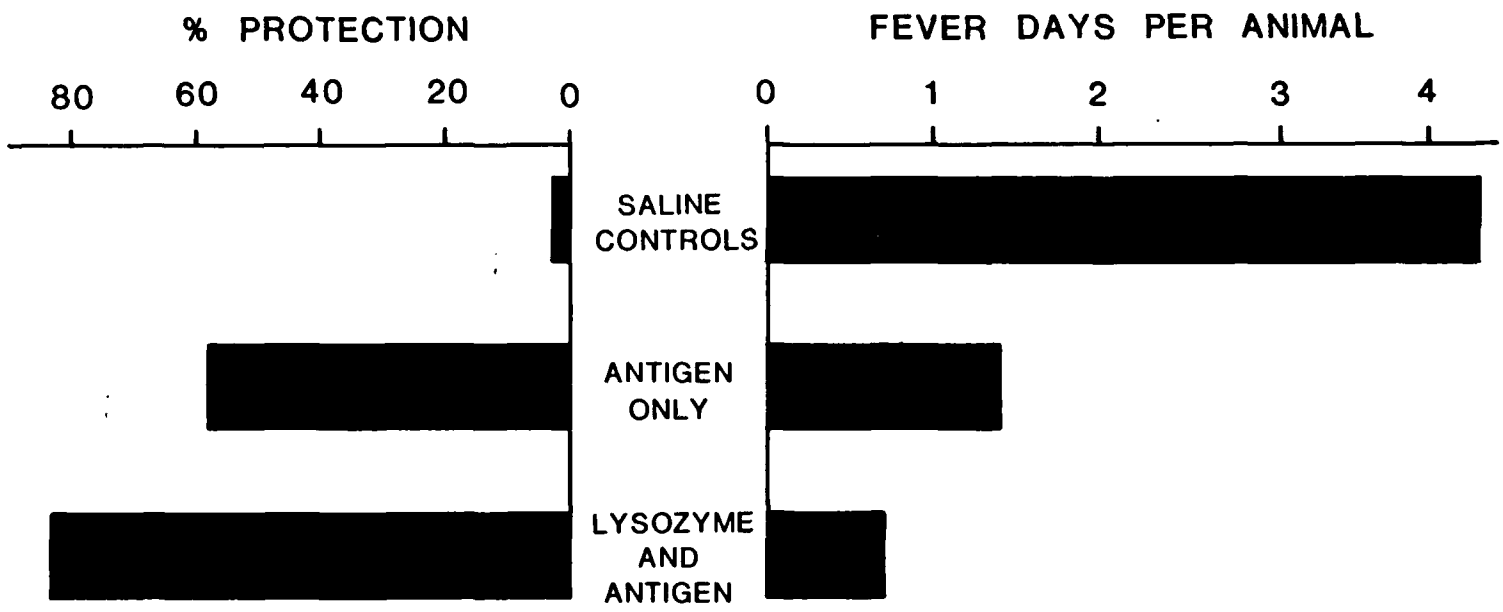
## FIGURE LEGENDS

FIG. 1. Effect of pretreatment of guinea pigs (n = 46) with lysozyme on protection against Q fever by phase I antigen of C. burnetii. Mean of 5 tests. Antigen ( μg protein) employed for first and second doses, respectively, for tests 1 through 5: 3.5, 3.5; 3.5, 7.0; 3.5, 14; 7.0, 7.0; 2.0 6.0. Lysozyme ( μg) employed for first and second doses, respectively, for tests 1 through 5: 12.5, 12.5; 25, 25; 12.5, 25; 12.5, 25; 50, 250.

FIG. 2. Effect of pretreatment of guinea pigs (n = 46) with lysozyme on antibody response to phase I antigen of C. burnetii. Mean of same 5 tests and same doses as for Figure 1.

FIG. 3. Migration inhibition of macrophages from guinea pigs vaccinated with phase I antigen of C. burnetii, with and without prior administration of lysozyme. (A) Antigen alone (n = 4). (B) Antigen + lysozyme (n = 4).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ANTIGEN ONLY
LYSOZYME AND ANTIGEN

| ANTIBODY | TREATMENT | | GEO. MEAN TITER |
|---|---|---|---|
| | ANTIGEN | LYSOZYME | |
| PHASE II CF | + | | 4.5 |
| | + | + | 12.2 |
| PHASE I MA | + | | 3.9 |
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| PHASE II MA | + | | 28.6 |
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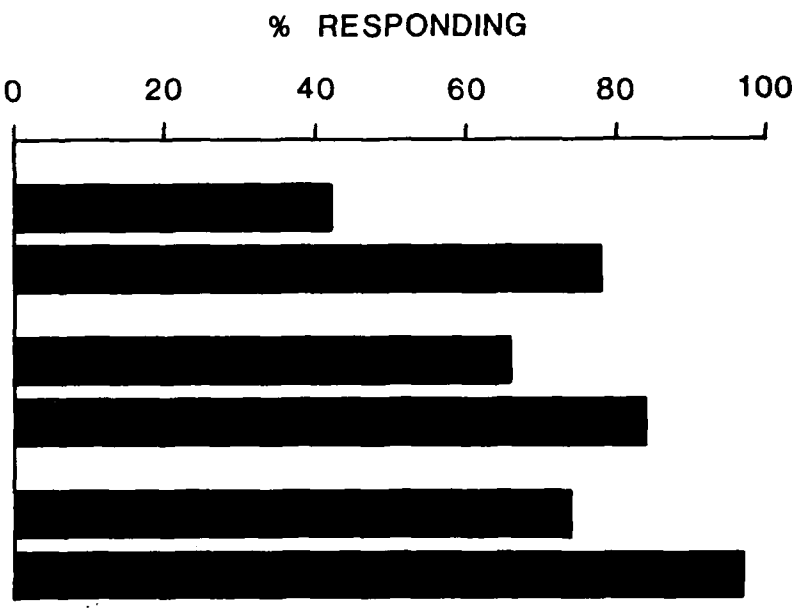

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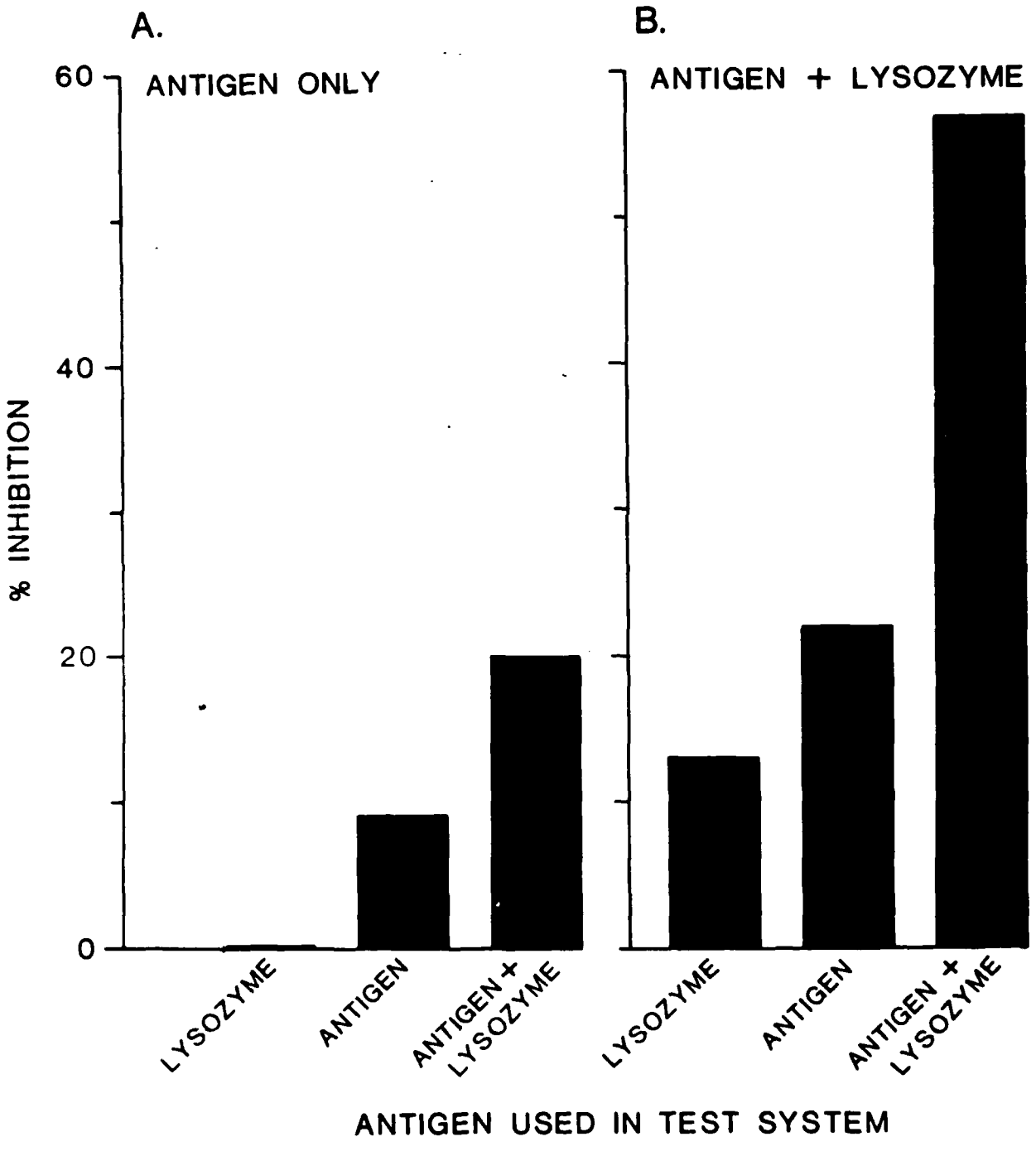
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